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# Sex Differences in Resting State Neural Networks of Nicotine-Dependent Cigarette Smokers

**Reagan R. Wetherill**[a,*], **Kanchana Jagannathan**[a], **Joshua Shin**[a], and **Teresa R. Franklin**[a]
[a] Department of Psychiatry, University of Pennsylvania, Philadelphia, PA, 19104, USA

## Abstract

Although several sex differences in nicotine dependence have been identified, the neural mechanisms underlying these sex differences are not clear. The present study examines sex differences in resting-state brain activity using an arterial spin labeling (ASL) perfusion imaging technique. Fifty-one (31 males) sated nicotine-dependent cigarette smokers underwent perfusion functional magnetic resonance imaging during the resting state. Using functionally defined hippocampus/amygdala (HIP/AMY) seed regions, we observed sex differences in correlation strength between the HIP/AMY and the bilateral anterior insula, rostral anterior cingulate cortex, and inferior parietal lobule with females showing stronger functional coupling than males. This pattern of synchronous variations in dynamic cerebral blood flow is consistent with recent models of nicotine dependence, and as such, our findings provide a novel perspective on the neural mechanisms that may contribute to sex differences in nicotine dependence.

### Keywords

Arterial spin labeling; Cerebral blood flow; Nicotine; Sex differences; Seed-based correlation analysis

## 1. INTRODUCTION

Preclinical and clinical research suggests that sex differences exist in all phases of nicotine dependence, including initiation, escalation of use, progression to addiction, withdrawal, and relapse (Becker & Hu, 2008; Lynch & Sofuoglu, 2010). For example, males have higher rates of past month cigarette smoking than females (Administration, 2012), yet females take less time to progress to dependence after initial use (Lynch, 2009), report shorter and less frequent abstinent periods (Pierce & Gilpin, 1996), find it more difficult to quit (Carpenter, Upadhyaya, LaRowe, Saladin, & Brady, 2006; Lynch, Roth, & Carroll, 2002), and appear to respond less favorably to smoking cessation treatments than males (Cepeda-Benito,



* Corresponding author: Reagan R. Wetherill, Ph.D. University of Pennsylvania, Department of Psychiatry 3900 Chestnut Street, Philadelphia, PA 19104, USA Telephone: (215) 222-3200 ext. 141 Fax: (215) 386-6770 rweth@mail.med.upenn.edu.

**Contributors**

Teresa R. Franklin designed and coordinated the study. Joshua Shin assisted in data collection. Kanchana Jagannathan analyzed the imaging data. Reagan R. Wetherill assisted in analyses and wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.



**Conflict of interest**

All authors declare that they have no conflicts of interest.

Reynoso, & Erath, 2004; Scharf & Shiffman, 2004). Although several sex differences in nicotine dependence have been identified, the mechanisms underlying these sex differences are not clear.

Differences in behavior are often associated with differences in neural functioning and network-level connectivity, and as such, functional magnetic resonance imaging (fMRI) has become a powerful tool in elucidating the neural differences underlying behavioral differences, like those observed in nicotine dependence. In a recent fMRI study, we examined sex differences in neural responses during smoking cue exposure relative to non-smoking cue exposure among sated nicotine-dependent cigarette smokers ($N$ =51; 31 males) and found that males showed greater smoking cue-induced neural activity than females in the bilateral hippocampus/amygdala (HIP/AMY) (Wetherill et al., 2013). The hippocampus and amygdala are structures associated with emotion, learning, and drug memories (Everitt & Robbins, 2005; Koob & Volkow, 2010). One potential explanation for our earlier findings is that female smokers may have stronger functional connections between reward- and memory-related brain regions, and therefore, require *less* neural activity in these brain regions when presented with smoking cues relative to males. We suggest that males and females may form distinct conditioned associations with smoking and neural responses to smoking cues, and consequently, may show sex-specific differences in HIP/AMY functional interactions.

Functional interactions between groups of brain regions (e.g. neural networks) can be observed by identifying synchronized spontaneous fluctuations in the blood oxygen level-dependent (BOLD) fMRI signal (Biswal, Yetkin, Haughton, & Hyde, 1995; Fox et al., 2005) or regional cerebral blood flow (CBF) (Zou, Wu, Stein, Zang, & Yang, 2009) in the absence of explicit task demands, or at rest. Indeed, resting-state functional connectivity (rsFC) approaches have identified specific brain networks that correspond to networks engaged during tasks (Smith et al., 2009) and predict behavioral performance (Kelly, Uddin, Biswal, Castellanos, & Milham, 2008). Furthermore, rsFC studies provide insight into the dysfunctional neurocircuitry underlying nicotine dependence. In a recent review of rsFC in addiction, Sutherland et al. (2012) provide a potential network model of nicotine addiction, which involves three distinct neural networks: 1) the default-mode network (DMN) (Raichle et al., 2001) comprised of the posterior cingulate, medial prefrontal cortices, and subcortical regions, 2) the executive control network (ECN) (Seeley et al., 2007), including lateral prefrontal and parietal regions involved in attention and decision making processes, and 3) the salience network (SN) (Seeley et al., 2007) anchored in the anterior cingulate cortex (ACC) and anterior insula and thought to influence information processing by identifying the most salient information both internally and externally, and "toggling" between the DMN and ECN (Uddin, Supekar, Ryali, & Menon, 2011). While this model provides a framework to potentially explain the neural processes underlying nicotine addiction, there are no studies examining sex differences within and between these neural networks, which could provide important information regarding inherent brain functioning differences between males and females that may contribute to sex differences in nicotine dependence.

To this end, we aimed to expand upon our previous research (Wetherill et al., 2013) by examining sex differences in rsFC of the HIP/AMY clusters that differed between males and females during smoking-related cue exposure. We hypothesized that HIP/AMY interactions with brain regions involved in salience (e.g., insula and ACC) and executive control ((e.g., inferior parietal lobule (IPL), dorsolateral prefrontal cortex (dlPFC)) would differ between males and females with females showing stronger functional coupling between these brain regions.

# 2. METHODS

## 2.1. Participants

Participants in the current study were previously reported on in a study examining sex differences in neural responses to smoking cues, and as such, were recruited and screened as described in Wetherill et al., 2013. Briefly, all eligible and interested participants provided informed consent and completed psychological and physical evaluations. Fifty-one physically healthy smokers (31 males) ranging in age from 18 to 58 years (34.2 ± 11.5) participated in the study. The sample is comprised of 69% Caucasians, 22% African Americans, and 9% Other/Mixed race. The study adhered to the Declaration of Helsinki and was approved by the University of Pennsylvania Institutional Review Board.

## 2.2. MR Acquisition and Processing

*Pseudo*-continuous arterial spin-labeled (*p*CASL) perfusion fMRI, a quantitative estimate of CBF and indirect measurement of neural activity (Floyd, Ratcliffe, Wang, Resch, & Detre, 2003), measured resting state CBF. Before the scanning session, participants smoked ad lib to minimize nicotine withdrawal-induced craving that might accrue during the scanning session. Scanning occurred approximately 25 minutes after smoking to allow the acute cardiovascular effects of smoking to dissipate. Participants completed the five minute *p*CASL resting baseline scan at the beginning of the scanning session.

Imaging data were acquired on a 3.0 Tesla Trio whole-body scanner (Siemens AG, Erlangen, Germany) using a Bruker volume coil (volume coils are designed to provide a homogenous receiving sensitivity and are 1 channel; Bruker Biospin, Billerica, MA) for 19 subjects and a standard 8-channel receive-only array head coil for the remaining 32 subjects. For co-registration of the functional data, a T1-weighted three-dimensional (3D) high resolution magnetization prepared rapid acquisition gradient echo (MPRAGE) scan was acquired with field of view (FOV)=160 mm, repetition time (TR)=1510 ms, echo time (TE)=3 ms, 192 × 256 matrix, slice thickness 1 mm for 19 subjects and FOV=250 mm, TR/TE=1620/3 ms, 192 × 256 matrix, slice thickness 1 mm for the remaining 32 subjects. *p*CASL perfusion fMRI sequence was used for resting baseline data acquisition. Interleaved images with and without labeling were obtained using a gradient echo echo-planar imaging sequence with a delay of 1000 ms for 19 subjects or 700 ms for 32 subjects inserted between the end of the labeling pulse and image acquisition (FOV = 130 mm, matrix = 64 × 64 × 14, TR/TE=3000/17 ms, flip angle=90°, slice thickness = 6 mm with a 2 mm inter-slice gap for 32 subjects and a 1.2 mm inter-slice gap for 19 subjects.

## 2.3. Data Processing and Analyses

Imaging data were analyzed using Statistical Parametric Mapping (SPM8; Wellcome Department of Cognitive Neurology, London, UK). An SPM-based arterial spin labeling (ASL) data processing toolbox (Wang et al., 2008) was used for *p*CASL perfusion data analyses. Briefly, ASL image pairs were realigned to the mean of all control images and spatially smoothed with a 3D isotropic Gaussian kernel at 10 mm full width at half maximum. For resting state data, 48 CBF image series were generated from the 48 label/control ASL image pairs using the same methods for CBF calculations. The mean control image of each subject's data was co-registered to the structural image using the mutual information based co-registration algorithm provided by SPM8. The same transformation parameters were applied to co-register the CBF maps to each subject's anatomical image. Subsequently, the structural image was spatially normalized to the Montreal Neurological Institute (MNI) standard brain. The resulting transformation matrix was used to align the CBF images to MNI space. A binary brain mask was used to exclude the non-brain areas in the CBF maps.

Correlation analysis, controlling for total intracranial volume, was used to examine sex differences in the temporal relationship between HIP/AMY seed regions and other brain regions. The functionally identified, bilateral HIP/AMY seed regions centered at [±20, −16, −15] were used based on our previous study showing sex differences in HIP/AMY neural responses to smoking cues (Wetherill et al., 2013). A cross-correlation coefficient (CC) map was obtained by correlating the average time course of the seed region with each voxel's time course over the brain. Adjustments for nuisance covariates (six head motion parameters and average time course retrieved from the segmented white matter mask) were made during the calculation of the CC map. The resulting correlation coefficients were converted to *z*-scores using Fisher's *r*-to-*z* transformation. The Z maps were then analyzed in a random-effects model in SPM8 to compare male and female connectivity. We identified regions showing differences in connectivity strength with a significant voxelwise statistical threshold ($p<0.005$) and, to control for multiple comparisons, voxels were required to be part of a cluster > 121 voxels, as determined by a Monte-Carlo simulation (*3dClustSim*, Analysis of Functional NeuroImages, http://afni.nimh.nih.gov) and resulted in 5% probability (corrected) of a cluster surviving due to chance.

## 3. RESULTS

Males were 36.2 (SD = 2.0) years old, and females were 30.9 (SD = 2.5) years old. There were no significant sex differences in age ($t_{49} = 1.69$, $p = 0.10$). Sex differences emerged for cigarettes smoked per day ($t_{49} = 2.12$, $p = 0.04$), with males smoking 16.9 (SD = 1.0) cigarettes per day and females smoking 13.6 (SD = 1.2) cigarettes per day. Participants reported smoking for 12.5 (SD = 1.6) years and had FTND scores of 4.5 (SD = 0.2), indicating moderate nicotine dependence.

In female smokers compared to male smokers, dynamic CBF fluctuations of the HIP/AMY at rest demonstrated higher correlations with CBF variations at rest in the bilateral anterior insula, rostral ACC, and left IPL (Figure 1, Table 1). There were no regions in which HIP/AMY dynamic CBF fluctuations showed decreased correlation with any other brain regions.

## 4. DISCUSSION

We examined the impact of sex on HIP/AMY resting-state functional connectivity and provide the first empirical support for sex differences in resting state networks of nicotine-dependent cigarette smokers. Using functionally defined bilateral HIP/AMY seeds, we observed sex differences in correlation strength of CBF fluctuations between the HIP/AMY and brain regions of two neural networks purported to be involved in nicotine dependence - the ECN and the SN (Sutherland, McHugh, Pariyadath, & Stein, 2012). Specifically, females demonstrated greater dynamic CBF variation coupling between the HIP/AMY and the bilateral anterior insula, the rostral ACC, and the IPL than males. These results provide a novel perspective on the neural mechanisms that may contribute to sex differences in nicotine-related behaviors and nicotine dependence.

The current findings expand upon our recent research showing that nicotine-dependent males showed greater neural responses to smoking cues (relative to non-smoking cues) in the bilateral HIP/AMY compared to females (Wetherill et al., 2013) by examining whether sex differences in rsFC could account for this finding. Given that females showed greater coupling between the HIP/AMY and reward- and memory-related brain regions than males, it is possible that females may be more efficient at processing and responding to smoking cues, and therefore, require *less* neural activation in these brain regions when presented with smoking cues relative to males. Thus, it appears that males and females may form distinct conditioned associations with smoking and neural responses to smoking cues, and

consequently, may show sex-specific differences in HIP/AMY functional interactions. While this interpretation is speculative, future research will explore this hypothesis.

It is important to note that the current data are among sated nicotine-dependent cigarette smokers, and as such, additional research is warranted in order to fully address the potential mechanisms underlying sex-dependent differences in nicotine dependence. Based on a recent rsFC study examining the effects of the smoking cessation aid, varenicline, and nicotine (alone and combined), rsFC varies according to state (i.e., has recently smoked a cigarette or is experiencing withdrawal) (Sutherland et al., 2013). Similarly, both preclinical and clinical literatures indicate that sex differences exist in response to nicotine administration and during withdrawal (Merritt, Cobb, & Cook, 2012; Pang & Leventhal, 2013). Together, these findings suggest that rsFC likely depends on whether the smoker is sated or in withdrawal and the smoker's biological sex.

### 4.1. Limitations

Our findings should be considered in light of the following limitations. A potential limitation in our findings could be due to differences in data acquisition. For example, 19 subjects were scanned using a Bruker coil; whereas, 32 subjects were scanned using an 8-channel coil. We examined whether data acquisition differences affected findings by comparing variances between groups using a homogeneity of variance test and found that the variances were not significantly different. This study is also limited in that we were unable to assess the influence of menstrual cycle phase/gonadal hormones due to insufficient sample sizes; however, we continue to acquire data to assess this in the future.

### 4.2. Conclusions

While preliminary, our findings highlight the utility of examining rsFC to elucidate the neural mechanisms underlying sex differences in nicotine dependence. Nicotine-dependent females showed greater HIP/AMY coupling with the bilateral anterior insula, the rostral ACC, and the IPL compared to nicotine-dependent males, which is consistent with neural network models of nicotine addiction (Janes, Nickerson, Frederick Bde, & Kaufman, 2012; Sutherland et al., 2012). Further, these findings extend previous research demonstrating sex differences in HIP/AMY neural responses to smoking cues (Wetherill et al., 2013) by identifying sex differences in rsFC among nicotine-dependent cigarette smokers that may account for the differences observed during smoking cue exposure. The current study contributes to our understanding of the neural mechanisms underlying sex differences in nicotine dependence, and ongoing research will help establish the link between these sex-specific neural features and subsequent smoking behavior and relapse.

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## HIGHLIGHTS

- Sex differences exist in nicotine dependence.
- We examined sex differences in resting-state functional connectivity of smokers.
- Females showed stronger coupling between resting neural networks than males.
- Findings may provide a mechanism underlying sex differences in nicotine dependence.

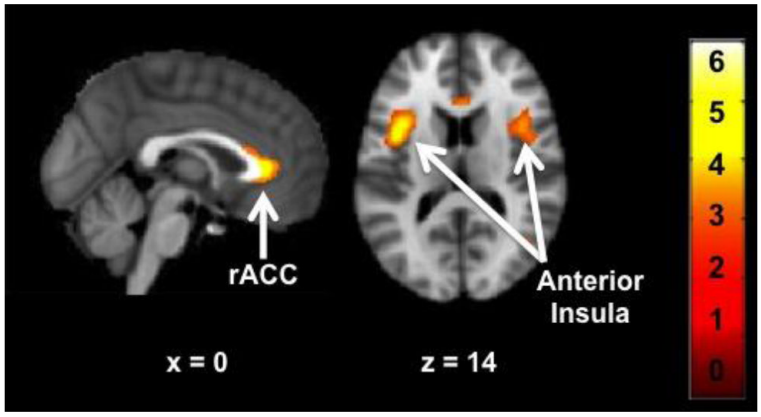


**Figure 1.**
Sex differences in the bilateral hippocampus/amygdala functional connectivity. All representations are positive functional connections with females > males. *T* values range from 3.47 to 4.40, corrected at $p < 0.005$. Images are displayed neurologically (left is left). rACC = rostral anterior cingulate cortex.

## Table 1

Regions showing increased connectivity with the hippocampus/amygdala (±20, −16, −15) in females compared to males.

| Region | Cluster size (voxels) | MNI coordinates | | | *T*-values |
|---|---|---|---|---|---|
| | | *x* | *y* | *z* | |
| Bilateral anterior insula | 525 | −40 | 6 | 14 | 4.40 |
| | 222 | 40 | 12 | 14 | 3.49 |
| Rostral ACC | 324 | 8 | 34 | 6 | 4.28 |
| Left IPL | 140 | −40 | −38 | 42 | 3.47 |

MNI coordinates and *T*-values of local maxima are shown for each cluster. Significant regions of interest level, $p < 0.005$, cluster corrected at $p < 0.05$ ($k > 121$ voxels). MNI = Montreal Neurological Institute, ACC = anterior cingulate cortex, IPL = inferior parietal lobule.